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1977

BIOASSAY OF
TOLBUTAMIDE
FOR POSSIBLE CARCINOGENICITY

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"Carcinogenesis technical report series

BIOASSAY OF

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Carcinogen Bioassay and Program Resources Branch
Carcinogenesis Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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Carcinogenesis Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health

CONTRIBUTORS: This report presents the results of the bioassay of tolbutamide for possible carcinogenicity, conducted for the Carcinogen Bioassay and Program Resources Branch, Carcinogenesis Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), Bethesda, Maryland. The bioassay was conducted by Southern Research Institute, Birmingham, Alabama, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI carcinogenesis bioassay program.

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Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[4]. Statistical analyses were performed by Dr. J. R. Joiner[5], using methods selected for the bioassay program by Dr. J. J. Gart[6]. Chemicals used in this bioassay were analyzed under the direction of Dr. E. Murrill[7], and analytical results were reviewed by Dr. C. W. Jameson[5].

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SUMMARY

A bioassay of tolbutamide for possible carcinogenicity was conducted by administering the test material in the diet to Fischer 344 rats and B6C3F1 mice.

Groups of 35 rats of each sex were administered tolbutamide at one of two doses, either 12,000 or 24,000 ppm, 5 days a week for 78 weeks, then observed for an additional 28 weeks. Matched-control groups consisted of 15 untreated rats of each sex. All surviving rats were killed at 106 or 107 weeks.

Groups of 35 mice of each sex were administered tolbutamide at one of two doses, either 25,000 or 50,000 ppm, 5 days a week for 78 weeks, then observed for an additional 24-26 weeks. Matched-control groups consisted of 15 untreated mice of each sex. All surviving mice were killed at 102-104 weeks.

Mean body weights of the treated rats and mice were lower than those of the corresponding matched controls during the entire study; however, survival was not significantly affected by treatment in either species. In both sexes of both species, survival was considered to be adequate for meaningful statistical analyses of the incidence of tumors.

In both the rats and the mice, a variety of neoplasms were found in both tolbutamide-treated and control groups. None of the neoplasms were present at a statistically significant increased incidence in treated groups of either species as compared with control groups and were not considered to be compound related.

It is concluded that under the conditions of this bioassay, tolbutamide was not carcinogenic for either Fischer 344 rats or B6C3F1 mice.

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I. INTRODUCTION

Tolbutamide (CAS 64-77-7; NCI C01763) was the first oral hypoglycemic agent used in the management of diabetes. It is one of the arylsulfonylurea hypoglycemics, a group which includes tolazamide, chlorpropamide, and acetohexamide. All of these compounds function by stimulating the secretion of insulin by the pancreas and, therefore, are used only in patients with at least minimal pancreatic function, as in maturity-onset diabetics (Larner and Haynes, 1975). Controlled studies have shown that the oral hypoglycemics may be no more effective than dietary modifications in controlling the symptoms of maturity-onset diabetes on a long-term basis and may be associated with an increase in cardiovascular mortality (Shen and Bressler, 1977).

Tolbutamide was selected for testing in the carcinogenesis program in an attempt to evaluate the carcinogenicity of certain drugs that are used extensively and for prolonged periods in humans.

II. MATERIALS AND METHODS

A. Chemical

Tolbutamide (1-butyl-3-(p-methylbenzenesulfonyl)urea) was obtained from the Upjohn Company, North Haven, Connecticut. The purity of Lot No. 656BC of tolbutamide used in the chronic study was determined to be 99.6 ± 0.6% by analysis at Midwest Research Institute. The melting point of this material was 127-129°C (literature: 126-127°C). Elemental analyses (C, H, N, S) were correct for $C_{12}H_{18}N_2O_3S$, the molecular formula of tolbutamide. The identity of the chemical was confirmed by nuclear magnetic resonance, infrared, and ultraviolet spectra, which were in agreement with the structure and matched the spectra given in the literature.

The batch of the chemical used for the chronic study was stored in a cold room at 5°C.

B. Dietary Preparation

Test diets containing tolbutamide were prepared every 2 weeks by mixing a known amount of sifted tolbutamide with a small amount of Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Ill.) in a portable mixer, then adding this mixture to the required amount of animal meal and mixing in a twin-shell blender for 10 minutes.

No analyses of concentration or determinations of stability of the chemical in feed were performed. The prepared diets were stored at room temperature in sealed plastic containers.

C. Animals

For the subchronic study, Swiss mice and Sprague-Dawley rats were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

For the chronic study, Fischer 344 rats were obtained from Harlan Industries, Cumberland, Indiana, and B6C3F1 mice were obtained from A. R. Schmidt, Madison, Wisconsin. These rats and mice were 30 days of age on arrival at the laboratory and were quarantined for an acclimation period (rats for 23 days, mice for 8 days). Animals with no clinical signs of disease were then assigned to control and treated groups and earmarked for individual identification.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature range was 20-24°C, and the relative humidity was maintained at 40-60%. There were 15 changes of room air per hour. The air was passed through both intake and exhaust fiberglass roughing filters. In addition to natural light, illumination was provided by fluorescent light for 9 hours per day. Food and water were supplied daily and available ad libitum.

Rats and mice were housed five per cage in solid-bottom stainless steel cages (Hahn Roofing and Sheet Metal Co., Birmingham, Ala.). The bottoms of the rat cages were lined with Iso-Dri® hardwood chips (Carworth, Edison, N.J.), and cage tops were covered with disposable filter bonnets; mouse cages were provided with Sterolit® clay bedding (Englehard Mineral and Chemical Co., New York, N.Y.) and covered with filter bonnets for the latter part of the chronic study. Bedding was replaced once per week; cages, water bottles, feeders, and racks were sanitized once per week.

The rats and mice were housed in separate rooms. Control animals were housed with respective treated animals. Animals treated with tolbutamide were maintained in the same rooms as animals of the same species being treated with the following chemicals:

RATS

Feed Studies

4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide
  (acetohexamide) (CAS 968-81-0)
anthranilic acid (CAS 118-92-3)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide
  (chlorpropamide) (CAS 94-20-2)
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine
  (pyrimethamine) (CAS 58-14-0)
2,6-diamino-3-(phenylazo)pyridine hydrochloride (CAS 136-40-3)
L-tryptophan (CAS 73-22-3)
N-9H-fluoren-2-ylacetamide (CAS 53-96-3)

N-(p-toluenesulfonyl)-N′-hexamethyleniminourea
  (tolazamide) (CAS 1156-19-0)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
pyrazinecarboxamide (pyrazinamide) (CAS 98-96-4)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
4,4′-thiodianiline (CAS 139-65-1)
ethionamide (CAS 536-33-4)

MICE

Feed Studies

4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide
  (acetohexamide) (CAS 968-81-0)
anthranilic acid (CAS 118-92-3)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide
  (chlorpropamide) (CAS 94-20-2)
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine
  (pyrimethamine) (CAS 58-14-0)
2,6-diamino-3-(phenylazo)pyridine hydrochloride (CAS 136-40-3)
L-tryptophan (CAS 73-22-3)
N-9H-fluoren-2-ylacetamide (CAS 53-96-3)
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea
  (tolazamide) (CAS 1156-19-0)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
pyrazinecarboxamide (pyrazinamide) (CAS 98-96-4)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
4,4′-thiodianiline (CAS 139-65-1)
ethionamide (CAS 536-33-4)

Gavage Studies

cholesterol (p-(bis(2-chloroethyl)amino)phenyl)acetate
  (phenesterin) (CAS 3546-10-9)
estradiol bis((p-(bis(2-chloroethyl)amino)phenyl)acetate)
  (estradiol mustard) (CAS 22966-79-6)

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride
  (MAAM) (NSC 141549)
acronine (CAS 7008-42-6)
5-azacytidine (CAS 320-67-2)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGDR)
  (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)

3,3′-iminobis-1-propanol dimethanesulfonate (ester)
hydrochloride (CAS 3458-22-8)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione
(ICRF-159) (CAS 21416-87-5)
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (isophosphamide) (CAS 3778-73-2)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine
hydrochloride (phenoxybenzamine) (CAS 63-92-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide
monohydrochloride (procarbazine) (CAS 366-70-1)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)
adriamycin (CAS 23214-92-8)

E. Subchronic Studies

Subchronic studies were conducted to estimate the maximum tolerated doses of tolbutamide, on the basis of which low and high concentrations (hereinafter referred to as "low doses" and "high doses") were determined for administration in the chronic studies. The drug was administered in feed at doses of 300, 800, 1,500, 3,000, or 6,000 ppm to male Sprague-Dawley rats; 12,000 or 24,000 ppm to female Sprague-Dawley rats; 500, 1,200, 2,500, 5,000, or 10,000 ppm to male Swiss mice; and 20,000 or 40,000 ppm to female Swiss mice. Treated animals received the test diets 7 days per week for 45 days and were observed for an additional 45 days. Five animals were treated at each dose, and 20 animals of each species were maintained as untreated controls.

No effects on body weight gain or survival and no gross abnormalities at autopsy were seen in the rats or mice at any dose tested. For rats, the low and high doses for the chronic studies were set at 12,000 and 24,000 ppm, respectively; for mice, they were set at 25,000 and 50,000 ppm.

F. Designs of Chronic Studies

The designs of the chronic studies are shown in tables 1 and 2.

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, and palpated for masses at each weighing. Rats and mice were weighed individually every 2 weeks for 80 weeks and once per month for the remainder of the study. Animals that died prior to day 100 were not necropsied, since it was assumed that they died of toxicity due to the test chemical. Animals that were moribund at the time of clinical examination were killed and necropsied.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were examined microscopically: skin, muscle, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder and bile duct (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid,

Table 1. Design of Tolbutamide Chronic Feeding Studies in Rats

Sex and Treatment Group	Initial No. of Animals[a]	Tolbutamide in Diet (ppm)[b]	Time on Study Treated (weeks)	Time on Study Untreated (weeks)
MALE				
Matched-Control	15	0		106
Low-Dose	35	12,000	78	28
High-Dose	35	24,000	78	28
FEMALE				
Matched-Control	15	0		107
Low-Dose	35	12,000	78	28
High-Dose	35	24,000	78	28

[a] Rats were 53 days of age when placed on study.

[b] The treated animals were fed the test diet 5 days per week and the control diet 2 days per week.

Table 2. Design of Tolbutamide Chronic Feeding Studies in Mice

Sex and Treatment Group	Initial No. of Animals[a]	Tolbutamide in Diet (ppm)[b]	Time on Study Treated (weeks)	Time on Study Untreated (weeks)
MALE				
Matched-Control	15	0		103
Low-Dose	35	25,000	78	25
High-Dose	35	50,000	78	24
FEMALE				
Matched-Control	15	0		103
Low-Dose	35	25,000	78	25-26
High-Dose	35	50,000	78	25

[a] Mice were 38 days of age when placed on study.

[b] The treated animals were fed the test diet 5 days per week and the control diet 2 days per week.

parathyroid, mammary gland, prostate or uterus, testis or ovary, brain, and sensory organs. Peripheral blood smears were prepared from each animal. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definitive diagnosis.

A few tissues from some animals were not examined, particularly from those animals that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic evaluation. Thus, the number of animals from which particular organs or tissues were examined microscopically varies, and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union

Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of treated animals at each dose level. When results for a number of treated groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups;

Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each treated group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

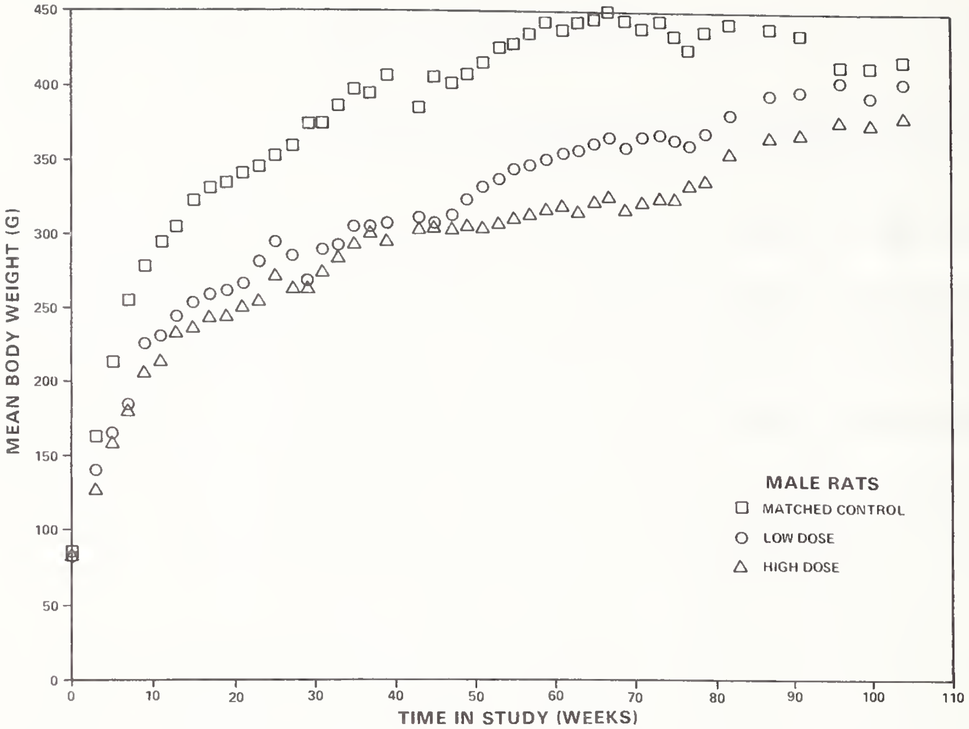
Mean body weights of rats of each sex at both the low and high doses were lower than those of the corresponding matched controls throughout the study (figure 1). The body weights of the high-dose groups were depressed more than those of the low-dose groups. Weight differentials became narrower after administration of tolbutamide was terminated, especially in the females.

No signs of treatment-related toxicity were reported in the rats. In an effort to control signs of respiratory disease, all rats in the colony were treated with oxytetracycline in the drinking water. Animals in this study were treated with oxytetracycline at 0.6 mg/ml in the drinking water during weeks 34-38, and at 0.3 mg/ml during weeks 38-40.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats fed tolbutamide in the diet at the doses of this experiment, together with those of the matched controls, are shown in figure 2.

In both sexes, the Tarone test results for positive dose-related trend in mortality over the period are not significant. In male



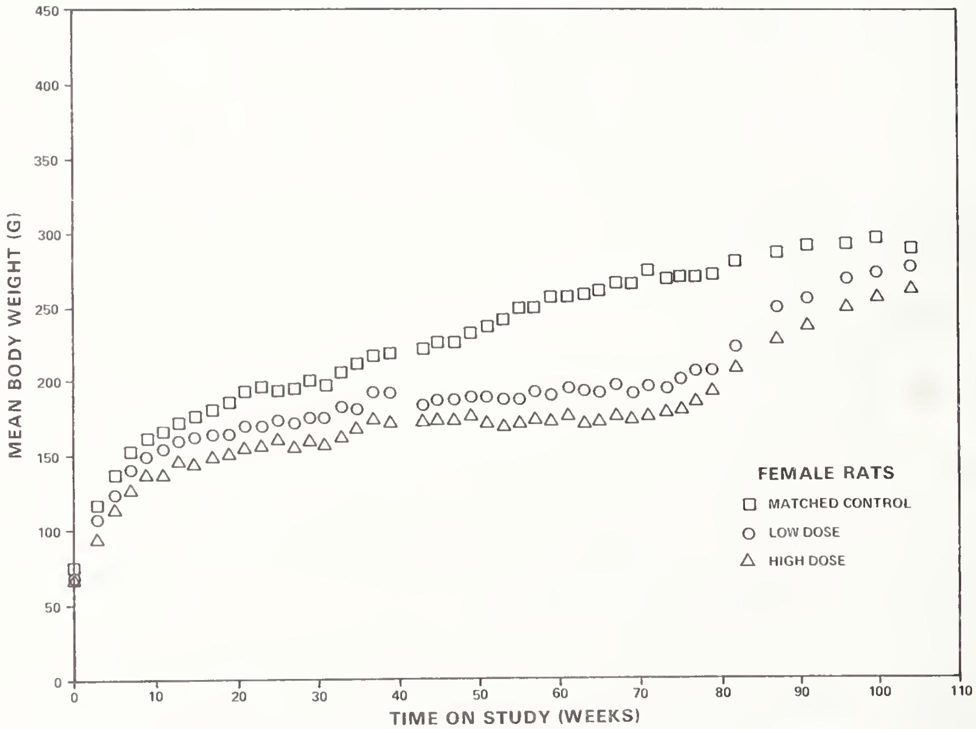


Figure 1. Growth Curves For Rats Fed Tolbutamide in the Diet

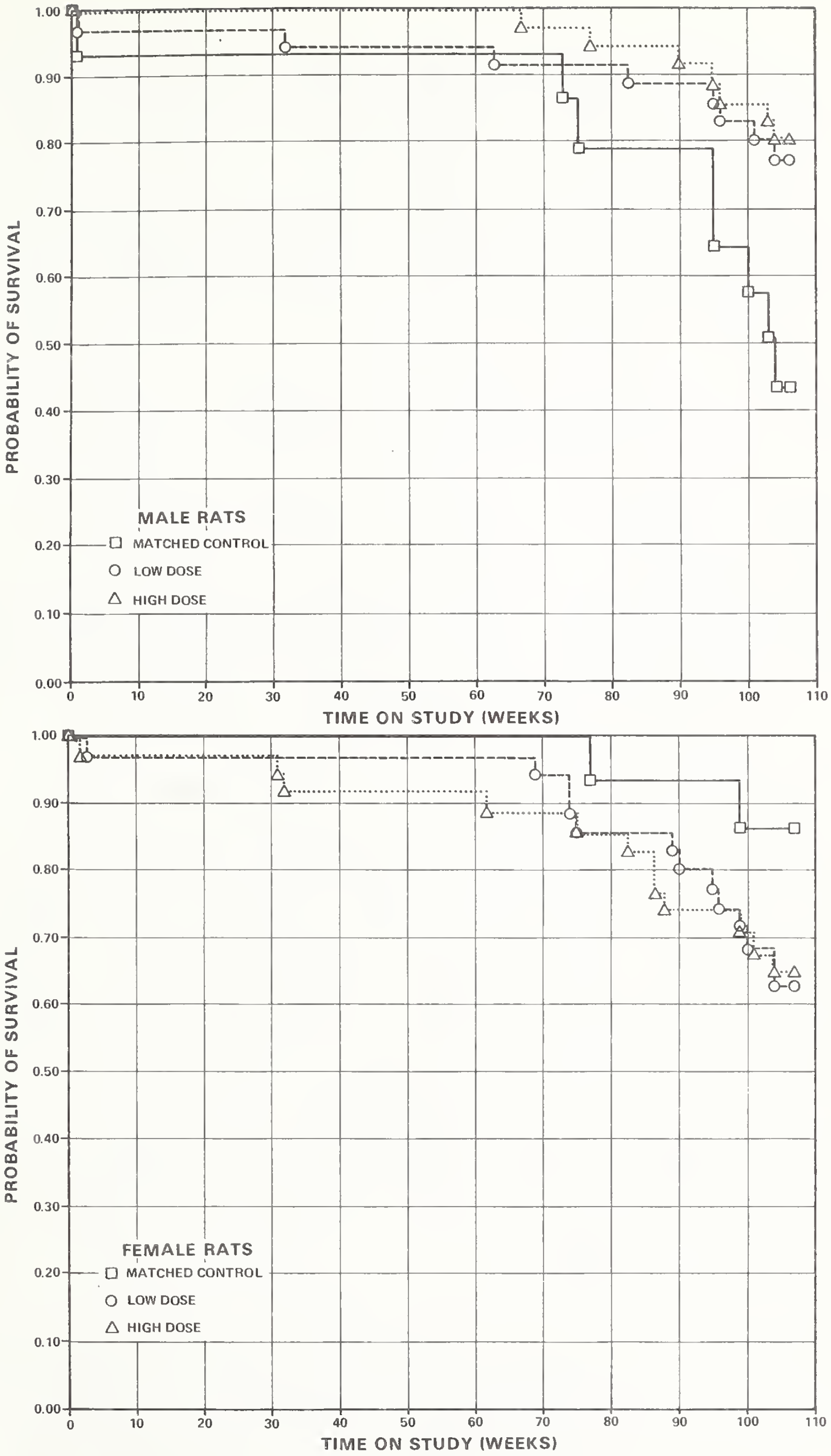


Figure 2. Survival Curves For Rats Fed Tolbutamide in the Diet

rats, 80% of the high-dose group, 77% of the low-dose group, and 43% of the matched-control group lived to the end of the study. In females, 65% of the high-dose group, 63% of the low-dose group, and 87% of the matched-control group survived to the end of the study. Sufficient numbers of rats of both sexes were available for meaningful statistical analyses of the incidences of late-developing tumors.

C. Pathology (Rats)

Histopathologic findings on neoplasms in rats are summarized in Appendix A, tables A1 and A2; findings on nonneoplastic lesions are summarized in Appendix C, tables C1 and C2.

There was a random occurrence of a variety of neoplasms in both the control and treated groups which were not considered to be compound related. The neoplasms listed in Appendix A appeared with approximately equal frequency in treated and control rats, or appeared in insignificant numbers. These lesions, however, are not uncommon in this strain of rat independent of any treatment. Few malignant tumors were observed, and no tumor metastases were recorded.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were encountered in animals of the control and treated groups (Appendix C). These nonneoplastic lesions are commonly seen in aged rats.

In the judgment of the pathologists, tolbutamide did not appear to induce neoplasms in Fischer 344 rats under the conditions of this bioassay.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidences of those specific primary tumors that were observed in at least 5% of one or more treated groups of either sex.

The results of the Cochran-Armitage test for positive dose-related trend and of the Fisher exact test for the direct comparison of incidences between the matched-control group and each of the treated groups in the positive direction are not significant in either sex. The incidences of mammary tumors in female rats exhibited a significant ($P < 0.001$) negative trend and both Fisher exact test results showed a significantly ($P < 0.001$) higher incidence in the controls than in either treated group. There were also significant results in the negative direction in the occurrence of thyroid tumors in male rats. In each of the 95% confidence intervals of relative risk, shown in the tables, a value of one or less than one is included; this indicates the absence of positive significant results. It should also be noted that some of the intervals have upper limits greater than one, indicating the theoretical possibility of the induction of tumors by tolbutamide, which could not be detected under the conditions of this test.

When groupings of types of tumors are made (as, for example, C-cell adenoma and carcinoma of the thyroid), the incidences of the individual components of the grouping are not included in the statistical analyses in the tables unless the proportions in any of the treated groups of either sex are 5% or more; however, a list of the incidences of each type of tumor is provided in tables A1 and A2 of Appendix A.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights for treated mice of both sexes were markedly lower than those of the corresponding matched controls during most or all of the tolbutamide administration period, and the degree of depression was the same in the low- and high-dose groups (figure 1). For unknown reasons, the surviving control male mice lost weight after week 70, and their weights gradually approximated those of the treated mice.

Except for effects on body weights, there were no signs of treatment-related toxicity in the mice. As a part of treatment of all mice at the laboratory to control signs of respiratory disease, mice in this study received oxytetracycline at 0.6 mg/ml in the drinking water during week 53, and 0.3 mg/ml during week 54. During weeks 52-63, the mouse room was treated with propylene glycol mist for the same purpose.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice fed tolbutamide in the diet at the doses of this experiment, together with those of the matched controls, are shown in figure 4.

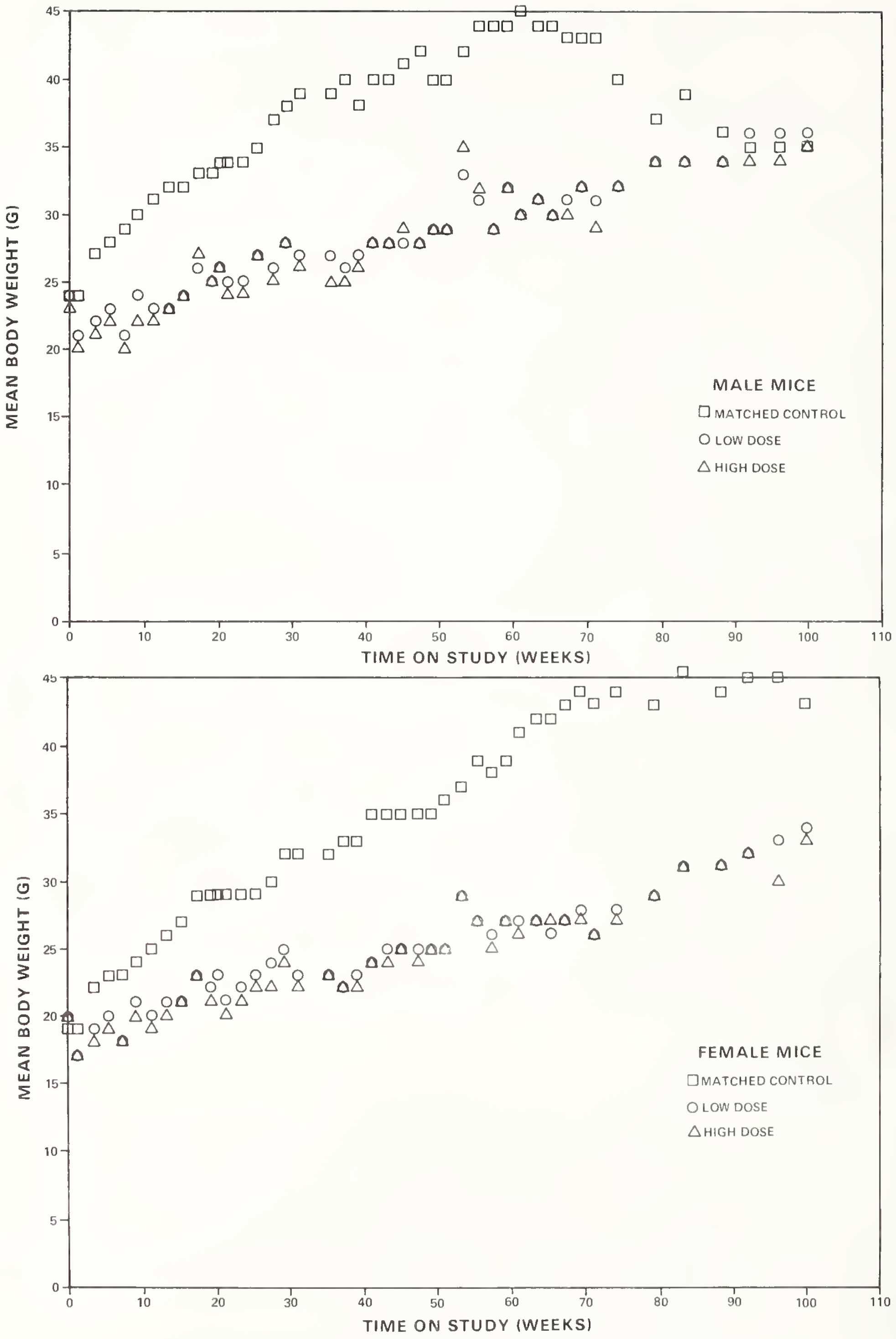


Figure 3. Growth Curves For Mice Fed Tolbutamide in the Diet

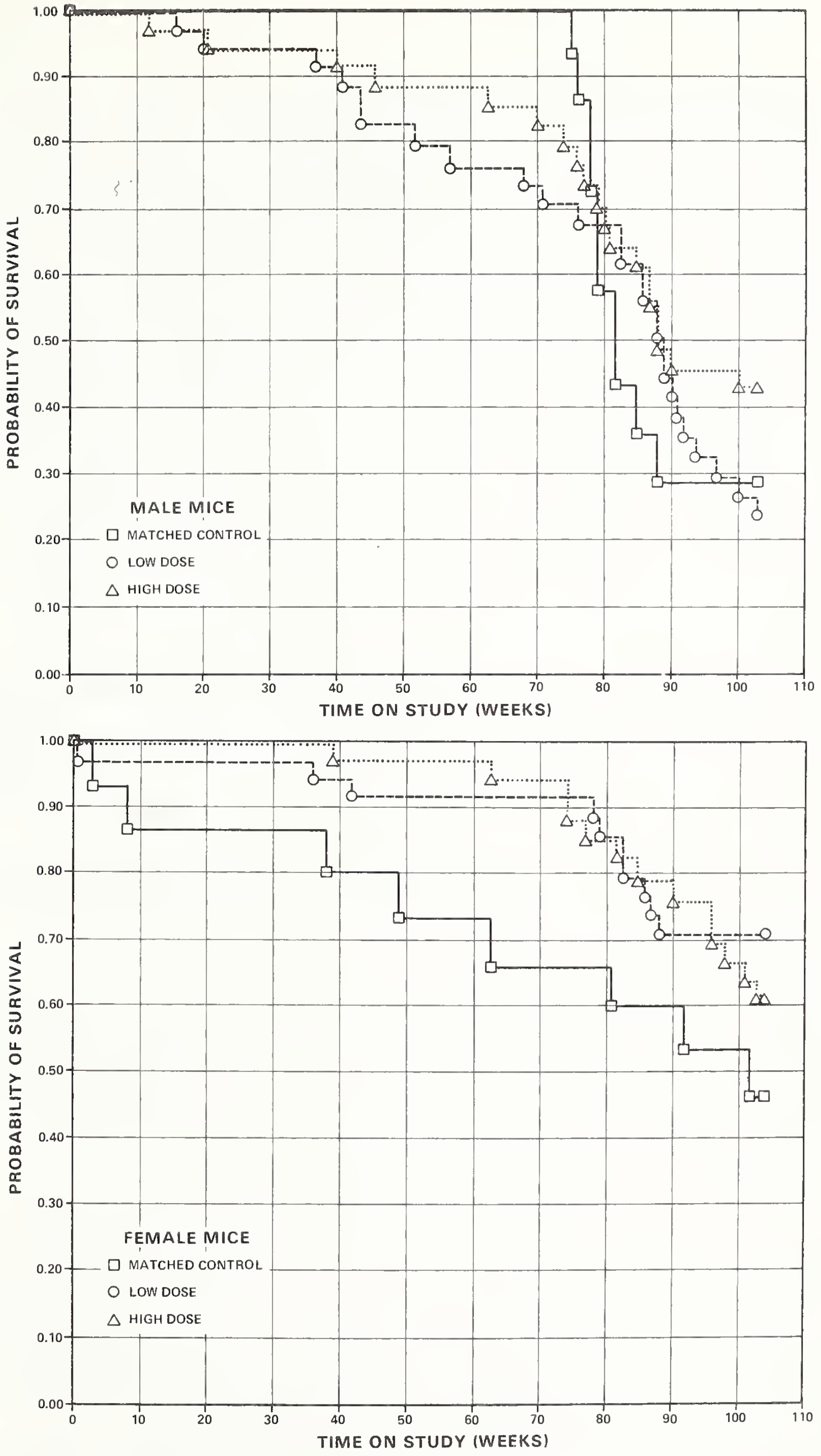


Figure 4. Survival Curves For Mice Fed Tolbutamide in the Diet

In neither sex were the results of the Tarone test significant for positive dose-related trend in mortality over the period of the bioassay. In male mice, 43% of the high-dose group, 24% of the low-dose group, and 29% of the matched-control group lived to the end of the study, and the respective median times on study were 88 weeks, 88 weeks, and 82 weeks. In the males, 29/35 high-dose, 28/35 low-dose, and all of the 15 control mice lived at least as long as 52 weeks. No tumor was observed before this time.

In females, 61% of the high-dose group, 71% of the low-dose group, and 47% of the matched-control group lived to the end of the study, providing an adequate numbers of mice for meaningful statistical analyses of the incidence of late-developing tumors.

C. Pathology (Mice)

Histopathologic findings on neoplasms in mice are summarized in Appendix B, tables B1 and B2; findings on nonneoplastic lesions are summarized in Appendix D, tables D1 and D2.

There was a random occurrence of a variety of neoplasms in both the control and treated groups which were not considered to be compound related. The tumors observed are not uncommon in this strain of mouse independent of any treatment.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were encountered in animals of the control and treated groups (Appendix D). These nonneoplastic lesions are commonly seen in aged mice; however, the suppurative lesions involving the lungs were associated with increased deaths or decreased life spans in the control and treated groups of male mice. The incidence of suppurative bronchopneumonia in the male mice was as follows: controls 8/14 (57%), low-dose 11/34 (32%), high-dose 18/32 (56%).

In the judgmemt of the pathologists, tolbutamide did not appear to induce neoplasms in B6C3F1 mice under the conditions of this bioassay.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those specific primary tumors that were observed in at least 5% of one or more treated groups of either sex.

The results of the Cochran-Armitage test for positive dose-related trend and of the Fisher exact test for the direct comparison of incidences between the matched-control group and each of the treated groups in the positive direction are not significant. The incidences of lymphomas and liver tumors in male mice had negative trend statistics of $P < 0.05$. In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one is included; this indicates the absence of positive significant results. It should also be noted that each of the intervals has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by tolbutamide, which could not be detected under the conditions of this test.

When chromophobe adenoma and carcinoma of the pituitary in female mice are combined into a grouping for analysis, the incidence of the combined lesions in treated animals as compared with controls is not statistically significant. A listing of each type of tumor is provided in tables B1 and B2 of Appendix B.

V. DISCUSSION

Tolbutamide administration resulted in decreased mean body weights of rats and mice of both sexes. The degree of weight depression was approximately equal regardless of the dose. Tests for dose-related trend in mortality were not significant in either sex of either species. Among rats, survival of both males and females was adequate for meaningful statistical analyses of the incidence of tumors. Among mice, survival at 106 weeks was 43% of the high-dose, 24% of the low-dose, and 29% of the matched-control males, and 61% of the high-dose, 71% of the low-dose, and 47% of the matched-control females. Adequate numbers of the female mice survived for meaningful statistical analyses of the incidence of tumors.

A variety of neoplasms were found in both the control and treated rats and mice. The incidences of these neoplasms were not statistically significant in rats or mice of either sex, and they were not considered to be compound related.

Tolbutamide is a sulfonylurea compound which has been used as an oral hypoglycemic agent for about 20 years. Long-term feeding studies in rats were reported by Bander (1959). Rats treated by oral gavage for 9 months at 250, 500, 1,000, and 2,000 mg/kg had slightly fatty livers and hyperplasia of thyroids only at the highest dose. No lesions attributable to treatment were observed in the tissues of dogs administered 100 mg/kg of tolbutamide by gavage for 9 months.

It is concluded that under the conditions of this bioassay, tolbutamide was not carcinogenic for Fischer 344 rats or B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS FED TOLBUTAMIDE IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED TOLBUTAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS MISSING	1		
ANIMALS NECROPSIED	13	34	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	13	34	34
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(13)	(34)	(34)
SARCOMA, NOS	1 (8%)		1 (3%)
FIBROMA		1 (3%)	1 (3%)
RESPIRATORY SYSTEM			
#LUNG	(13)	(33)	(34)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (3%)	1 (3%)
SARCOMA, NOS			1 (3%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(13)	(34)	(34)
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (3%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (8%)	2 (6%)	
UNDIFFERENTIATED LEUKEMIA	1 (8%)	2 (6%)	3 (9%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			
URINARY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(11)	(31)	(26)
CHROMOPHOBE ADENOMA	2 (18%)	5 (16%)	5 (19%)
#ADRENAL	(12)	(33)	(33)
PHEOCHROMOCYTOMA	1 (8%)		1 (3%)
#THYROID	(13)	(33)	(32)
FOLLICULAR-CELL CARCINOMA		1 (3%)	
C-CELL ADENOMA	4 (31%)	1 (3%)	3 (9%)
C-CELL CARCINOMA			1 (3%)
#PANCREATIC ISLETS	(13)	(33)	(32)
ISLET-CELL ADENOMA		1 (3%)	2 (6%)
REPRODUCTIVE SYSTEM			
#TESTIS	(13)	(34)	(33)
INTERSTITIAL-CELL TUMOR	11 (85%)	21 (62%)	22 (67%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(13)	(34)	(34)
OSTEOSARCOMA	1 (8%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	2	3	3
MORIBUND SACRIFICE	6	5	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	6	27	28
ANIMAL MISSING	1		

@ INCLUDES AUTOLYZED ANIMALS

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	24	27
TOTAL PRIMARY TUMORS	22	36	41
TOTAL ANIMALS WITH BENIGN TUMORS	12	24	25
TOTAL BENIGN TUMORS	18	30	35
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	6	6
TOTAL MALIGNANT TUMORS	4	6	6
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED TOLBUTAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS MISSING			1
ANIMALS NECROPSIED	14	31	31
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	31	31
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(14)	(31)	(31)
ADNEXAL CARCINOMA			1 (3%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(14)	(31)	(31)
MALIG.LYMPHOMA, UNDIFFER-TYPE		2 (6%)	1 (3%)
UNDIFFERENTIATED LEUKEMIA	2 (14%)	3 (10%)	2 (6%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			
URINARY SYSTEM			
#URINARY BLADDER	(14)	(30)	(27)
PAPILLOMA, NOS		1 (3%)	1 (4%)
TRANSITIONAL-CELL PAPILLOMA		1 (3%)	
ENDOCRINE SYSTEM			
#PITUITARY	(12)	(30)	(23)
CHROMOPHOBE ADENOMA	5 (42%)	8 (27%)	11 (48%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#THYROID	(14)	(31)	(30)
C-CELL ADENOMA	2 (14%)		
C-CELL CARCINOMA	1 (7%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(14)	(31)	(31)
ADENOCARCINOMA, NOS		1 (3%)	
FIBROADENOMA	8 (57%)	2 (6%)	
*PREPUTIAL GLAND	(14)	(31)	(31)
ADNEXAL CARCINOMA	1 (7%)		
*VAGINA	(14)	(31)	(31)
SARCOMA, NOS		1 (3%)	
#UTERUS	(14)	(30)	(30)
SARCOMA, NOS			1 (3%)
ENDOMETRIAL STROMAL POLYP	4 (29%)	2 (7%)	2 (7%)
#UTERUS/ENDOMETRIUM	(14)	(30)	(30)
ADENOCARCINOMA, NOS	1 (7%)		
PAPILLARY ADENOCARCINOMA		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	2	6	7
MORIBUND SACRIFICE		7	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	13	22	22
ANIMAL MISSING			1

@ INCLUDES AUTOLYZED ANIMALS

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	13	15	16
TOTAL PRIMARY TUMORS	24	22	19
TOTAL ANIMALS WITH BENIGN TUMORS	12	10	13
TOTAL BENIGN TUMORS	19	14	14
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	7	5
TOTAL MALIGNANT TUMORS	5	8	5
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE FED TOLBUTAMIDE IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED TOLBUTAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS MISSING	1	1	
ANIMALS NECROPSIED	14	34	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	34	32
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(14)	(34)	(32)
SARCOMA, NOS	1 (7%)		
FIBROSARCOMA		1 (3%)	
RESPIRATORY SYSTEM			
#LUNG	(14)	(34)	(32)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (3%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (7%)	1 (3%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(14)	(34)	(32)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (7%)	5 (15%)	
MALIGNANT LYMPHOMA, MIXED TYPE	1 (7%)	1 (3%)	
MALIGNANT RETICULOSIS		1 (3%)	
#MEDIASTINAL L.NODE	(14)	(25)	(21)
ALVEOLAR/BRONCHIOLAR CA, METASTA		1 (4%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(14)	(34)	(32)
HEPATOCELLULAR ADENOMA	2 (14%)	2 (6%)	1 (3%)
HEPATOCELLULAR CARCINOMA	2 (14%)	2 (6%)	
HEMANGIOMA			1 (3%)
URINARY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(12)	(30)	(18)
CHROMOPHOBE ADENOMA		1 (3%)	
#THYROID	(14)	(29)	(24)
FOLLICULAR-CELL ADENOMA	1 (7%)	1 (3%)	
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(14)	(34)	(32)
ADENOMA, NOS	2 (14%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	4	10	9
MORIBUND SACRIFICE	6	16	10
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			2
TERMINAL SACRIFICE	4	8	14
ANIMAL MISSING	1	1	

@ INCLUDES AUTOLYZED ANIMALS

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	8	13	2
TOTAL PRIMARY TUMORS	11	16	2
TOTAL ANIMALS WITH BENIGN TUMORS	4	5	2
TOTAL BENIGN TUMORS	5	5	2
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	9	
TOTAL MALIGNANT TUMORS	6	11	
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE FED TOLBUTAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	12	34	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	12	34	34
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(12)	(34)	(34)
HEMANGIOSARCOMA	1 (8%)		
RESPIRATORY SYSTEM			
#LUNG	(11)	(34)	(34)
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (6%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(12)	(34)	(34)
MALIGNANT LYMPHOMA, NOS		1 (3%)	
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (3%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		2 (6%)	
#PEYERS PATCH	(11)	(33)	(33)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(12)	(34)	(34)
HEPATOCELLULAR ADENOMA		2 (6%)	1 (3%)
HEPATOCELLULAR CARCINOMA	1 (8%)		
URINARY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(11)	(26)	(26)
CHROMOPHOBE ADENOMA	1 (9%)		
CHROMOPHOBE CARCINOMA		2 (8%)	
#ADRENAL	(11)	(34)	(34)
PHEOCHROMOCYTOMA, MALIGNANT		1 (3%)	
#THYROID	(12)	(25)	(32)
FOLLICULAR-CELL ADENOMA	1 (8%)		
REPRODUCTIVE SYSTEM			
#UTERUS	(11)	(34)	(34)
SARCOMA, NOS	1 (9%)	1 (3%)	
#OVARY	(11)	(34)	(34)
PAPILLARY ADENOMA			1 (3%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(12)	(34)	(34)
ADENOMA, NOS			1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	5	7	4
MORIBUND SACRIFICE	3	3	9
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		1	2
TERMINAL SACRIFICE	7	24	20
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	3	11	3
TOTAL PRIMARY TUMORS	5	13	3
TOTAL ANIMALS WITH BENIGN TUMORS	2	4	3
TOTAL BENIGN TUMORS	2	4	3
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	9	
TOTAL MALIGNANT TUMORS	3	9	
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS FED TOLBUTAMIDE IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED TOLBUTAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS MISSING	1		
ANIMALS NECROPSIED	13	34	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	13	34	34
INTEGUMENTARY SYSTEM			
*SKIN	(13)	(34)	(34)
EPIDERMAL INCLUSION CYST	1 (8%)	2 (6%)	4 (12%)
RESPIRATORY SYSTEM			
#TRACHEA	(13)	(34)	(34)
LYMPHOCYTIC INFILTRATE		1 (3%)	1 (3%)
INFLAMMATION, SUPPURATIVE	2 (15%)		
#LUNG/BRONCHUS	(13)	(33)	(34)
HYPERPLASIA, LYMPHOID		6 (18%)	
#LUNG/BRONCHIOLE	(13)	(33)	(34)
HYPERPLASIA, LYMPHOID			1 (3%)
#LUNG	(13)	(33)	(34)
BRONCHOPNEUMONIA SUPPURATIVE		1 (3%)	
PNEUMONIA, CHRONIC MURINE		6 (18%)	
FIBROSIS, FOCAL		1 (3%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(12)	(33)	(31)
ATROPHY, NOS			1 (3%)
MYELOFIBROSIS		1 (3%)	
#SPLEEN	(13)	(33)	(34)
HYPERPLASIA, RETICULUM CELL			1 (3%)
HEMATOPOIESIS			1 (3%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(13)	(34)	(34)
INFLAMMATION, INTERSTITIAL	4 (31%)	5 (15%)	8 (24%)

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
*BILE DUCT	(13)	(34)	(34)
HYPERPLASIA, FOCAL	1 (8%)		
#PANCREAS	(13)	(33)	(32)
INFLAMMATION, INTERSTITIAL		1 (3%)	2 (6%)
INFLAMMATION, CHRONIC	1 (8%)	3 (9%)	1 (3%)
INFLAMMATION, CHRONIC FOCAL		2 (6%)	1 (3%)
PERIARTERITIS		1 (3%)	
#STOMACH	(13)	(33)	(33)
MINERALIZATION	1 (8%)		
URINARY SYSTEM			
#KIDNEY	(12)	(33)	(33)
INFLAMMATION, CHRONIC	2 (17%)	5 (15%)	3 (9%)
INFLAMMATION, CHRONIC DIFFUSE	7 (58%)	4 (12%)	9 (27%)
#KIDNEY/TUBULE	(12)	(33)	(33)
MINERALIZATION	1 (8%)		
ENDOCRINE SYSTEM			
#THYROID	(13)	(33)	(32)
HYPERPLASIA, C-CELL	1 (8%)	1 (3%)	
REPRODUCTIVE SYSTEM			
#PROSTATE	(11)	(32)	(33)
INFLAMMATION, SUPPURATIVE			1 (3%)
NERVOUS SYSTEM			
#CEREBRUM	(12)	(33)	(34)
HEMORRHAGE		1 (3%)	
*SPINAL CORD	(13)	(34)	(34)
MALACIA			1 (3%)

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EYE/RETINA	(13)	(34)	(34)
ATROPHY, NOS		1 (3%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(13)	(34)	(34)
NECROSIS, FAT	1 (8%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	2	2
ANIMAL MISSING/NO NECROPSY	1		
NO NECROPSY PERFORMED	1	1	
AUTOLYSIS/NO NECROPSY			1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED TOLBUTAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS MISSING			1
ANIMALS NECROPSIED	14	31	31
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	31	31
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(14)	(31)	(31)
LYMPHOCYTIC INFILTRATE		1 (3%)	
INFLAMMATION, SUPPURATIVE	1 (7%)	2 (6%)	
#LUNG/BRONCHUS	(14)	(31)	(31)
HYPERPLASIA, LYMPHOID			1 (3%)
#LUNG	(14)	(31)	(31)
PNEUMONIA, CHRONIC MURINE			3 (10%)
FIBROSIS			1 (3%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(13)	(29)	(29)
MYELOFIBROSIS			1 (3%)
#SPLEEN	(14)	(31)	(30)
HYPERPLASIA, RETICULUM CELL		1 (3%)	
HEMATOPOIESIS			2 (7%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(14)	(30)	(30)
INFLAMMATION, INTERSTITIAL		2 (7%)	2 (7%)
DIGESTIVE SYSTEM			
#LIVER	(14)	(31)	(30)
HYPERPLASIA, NODULAR		1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(14)	(31)	(31)
INFLAMMATION, CHRONIC	1 (7%)		
INFLAMMATION, CHRONIC DIFFUSE		1 (3%)	1 (3%)
ENDOCRINE SYSTEM			
#THYROID	(14)	(31)	(30)
CYSTIC FOLLICLES		1 (3%)	
HYPERPLASIA, C-CELL		1 (3%)	
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(14)	(31)	(31)
HYPERPLASIA, EPITHELIAL		1 (3%)	
#UTERUS/ENDOMETRIUM	(14)	(30)	(30)
INFLAMMATION, SUPPURATIVE	1 (7%)		1 (3%)
HYPERPLASIA, CYSTIC		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/RETINA	(14)	(31)	(31)
ATROPHY, NOS	1 (7%)		
*LENS CAPSULE	(14)	(31)	(31)
MINERALIZATION		1 (3%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(14)	(31)	(31)
FIBROSIS			1 (3%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	11	9
ANIMAL MISSING/NO NECROPSY			1
NO NECROPSY PERFORMED		1	1
AUTOLYSIS/NO NECROPSY	1	3	2

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE FED TOLBUTAMIDE IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FEDTOLBUTAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS MISSING	1	1	
ANIMALS NECROPSIED	14	34	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	34	32
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(14)	(34)	(31)
INFLAMMATION, SUPPURATIVE	1 (7%)	7 (21%)	3 (10%)
#LUNG/BRONCHIOLE	(14)	(34)	(32)
HYPERPLASIA, PLASMA CELL			3 (9%)
#LUNG	(14)	(34)	(32)
BRONCHOPNEUMONIA SUPPURATIVE	8 (57%)	10 (29%)	18 (56%)
BRONCHOPNEUMONIA CHRONIC SUPPURA		1 (3%)	
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
#MYOCARDIUM	(14)	(34)	(32)
INFLAMMATION, INTERSTITIAL			1 (3%)
INFLAMMATION, CHRONIC DIFFUSE			1 (3%)
DIGESTIVE SYSTEM			
#LIVER	(14)	(34)	(32)
HEMORRHAGE	1 (7%)		
HEMORRHAGE, CHRONIC	1 (7%)		
NECROSIS, COAGULATIVE		3 (9%)	

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NODULAR			1 (3%)
#LIVER/CENTRILOBULAR	(14)	(34)	(32)
NECROSIS, COAGULATIVE	1 (7%)		
URINARY SYSTEM			
#KIDNEY	(14)	(34)	(31)
HYDRONEPHROSIS			1 (3%)
PYELONEPHRITIS SUPPURATIVE		1 (3%)	
#KIDNEY/CORTEX	(14)	(34)	(31)
NECROSIS, COAGULATIVE		1 (3%)	
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(14)	(34)	(32)
ULCER, CHRONIC		1 (3%)	
*EYE/CONJUNCTIVA	(14)	(34)	(32)
ULCER, CHRONIC		1 (3%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		9	11
ANIMAL MISSING/NO NECROPSY	1	1	
ACCIDENTAL DEATH			1
NO NECROPSY PERFORMED			1
AUTOLYSIS/NO NECROPSY			1

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED TOLBUTAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	12	34	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	12	34	34
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(12)	(34)	(34)
INFLAMMATION, SUPPURATIVE	3 (25%)		6 (18%)
#LUNG/BRONCHUS	(11)	(34)	(34)
INFLAMMATION, SUPPURATIVE			1 (3%)
#LUNG	(11)	(34)	(34)
BRONCHOPNEUMONIA SUPPURATIVE	4 (36%)	1 (3%)	11 (32%)
BRONCHOPNEUMONIA CHRONIC SUPPURA			1 (3%)
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
#MYOCARDIUM	(11)	(34)	(34)
INFLAMMATION, INTERSTITIAL		1 (3%)	
DIGESTIVE SYSTEM			
#LIVER	(12)	(34)	(34)
HYPERPLASIA, NODULAR			1 (3%)
URINARY SYSTEM			
#KIDNEY	(12)	(34)	(34)
HYDRONEPHROSIS			2 (6%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#UTERUS/ENDOMETRIUM	(11)	(34)	(34)
INFLAMMATION, SUPPURATIVE		1 (3%)	1 (3%)
#OVARY	(11)	(34)	(34)
HEMORRHAGE, CHRONIC		1 (3%)	
INFLAMMATION, CHRONIC SUPPURATIV	1 (9%)		
NERVOUS SYSTEM			
#BRAIN	(12)	(34)	(34)
PERIVASCULAR CUFFING		2 (6%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC FOCAL	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3	20	16

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NO NECROPSY PERFORMED	2	1	
AUTOLYSIS/NO NECROPSY	1		1

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS FED TOLBUTAMIDE IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Tolbutamide in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia[b]	2/13 (15)	5/34 (15)	3/34 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.956	0.574
Lower Limit		0.188	0.077
Upper Limit		9.325	6.438
Weeks to First Observed Tumor	95	95	90
Pituitary: Chromophobe Adenoma[b]	2/11 (18)	5/31 (16)	5/26 (19)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.887	1.058
Lower Limit		0.183	0.217
Upper Limit		8.485	9.996
Weeks to First Observed Tumor	100	106	104

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Tolbutamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma[b]	4/13 (31)	1/33 (3)	3/32 (9)
P Values[c,d]	N.S.	P = 0.018 (N)	N.S.
Departure from Linear Trend[e]	P = 0.019		
Relative Risk (Matched Control)[f]		0.098	0.305
Lower Limit		0.002	0.055
Upper Limit		0.895	1.593
Weeks to First Observed Tumor	103	106	104
Thyroid: C-cell Adenoma or Carcinoma[b]	4/13 (31)	1/33 (3)	4/32 (13)
P Values[c,d]	N.S.	P = 0.018 (N)	N.S.
Departure from Linear Trend[e]	P = 0.015		
Relative Risk (Matched Control)[f]		0.098	0.406
Lower Limit		0.002	0.095
Upper Limit		0.895	1.914
Weeks to First Observed Tumor	103	106	104

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Tolbutamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor[b]	11/13 (85)	21/34 (62)	22/33 (67)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.730	0.788
Lower Limit		0.579	0.628
Upper Limit		1.205	1.280
Weeks to First Observed Tumor	75	96	95

[a] Treated groups received doses of 12,000 or 24,000 ppm in feed.

[b] Number of tumor-bearing animals/number of animals examined at site (percent).

[c] Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$, otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d] A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e] The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f] The 95% confidence interval of the relative risk between each treated group and the control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Tolbutamide in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Leukemia[b]	2/14 (14)	3/31 (10)	2/31 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.677	0.452
Lower Limit		0.091	0.037
Upper Limit		7.510	5.825
Weeks to First Observed Tumor	107	69	87
Hematopoietic System: Lymphoma[b]	0/14 (0)	2/31 (6)	1/31 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.142	0.026
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	100	106

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Tolbutamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia[b]	2/14 (14)	5/31 (16)	3/31 (10)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		1.129	0.677
Lower Limit		0.221	0.091
Upper Limit		11.053	7.510
Weeks to First Observed Tumor	107	69	87
Pituitary: Chromophobe Adenoma[b]	5/12 (42)	8/30 (27)	11/23 (48)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.640	1.148
Lower Limit		0.253	0.511
Upper Limit		2.079	3.308
Weeks to First Observed Tumor	107	96	87

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Tolbutamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma[b]	2/14 (14)	0/31 (0)	0/30 (0)
P Values[c,d]	P = 0.031 (N)	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		2.276	2.336
Weeks to First Observed Tumor	107	--	--
Thyroid: C-cell Adenoma or Carcinoma[b]	3/14 (21)	0/31 (0)	0/30 (0)
P Values[c,d]	P = 0.006 (N)	P = 0.026 (N)	P = 0.027 (N)
Departure from Linear Trend[e]	P = 0.022		
Relative Risk (Matched Control)[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.011	1.042
Weeks to First Observed Tumor	107	--	--

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Tolbutamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	8/14 (57)	2/31 (6)	0/31 (0)
P Values[c,d]	P < 0.001 (N)	P < 0.001 (N)	P < 0.001 (N)
Departure from Linear Trend[e]	P = 0.006		
Relative Risk (Matched Control)[f]		0.113	0.000
Lower Limit		0.015	0.000
Upper Limit		0.479	0.234
Weeks to First Observed Tumor	99	106	--
Uterus: Endometrial Stromal Polyp[b]	4/14 (29)	2/30 (7)	2/30 (7)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.233	0.233
Lower Limit		0.024	0.024
Upper Limit		1.452	1.452
Weeks to First Observed Tumor	107	106	106

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Tolbutamide in the Diet[a]

(continued)

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[a] Treated groups received doses of 12,000 or 24,000 ppm in feed.

[b] Number of tumor-bearing animals/number of animals examined at site (percent).

[c] Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$, otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d] A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e] The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f] The 95% confidence interval of the relative risk between each treated group and the control group.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE FED TOLBUTAMIDE IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed Tolbutamide in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma[b]	2/14 (14)	6/34 (18)	0/32 (0)
P Values[c,d]	P = 0.046 (N)	N.S.	N.S.
Relative Risk (Matched Control)[f]		1.235	0.000
Lower Limit		0.266	0.000
Upper Limit		11.532	2.200
Weeks to First Observed Tumor	103	76	--
Liver: Hepatocellular Adenoma[b]	2/14 (14)	2/34 (6)	1/32 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.412	0.219
Lower Limit		0.034	0.004
Upper Limit		5.351	3.927
Weeks to First Observed Tumor	88	88	102

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed Tolbutamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	2/14 (14)	2/34 (6)	0/32 (0)
P Values[c,d]	P = 0.045 (N)	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.412	0.000
Lower Limit		0.034	0.000
Upper Limit		5.351	2.200
Weeks to First Observed Tumor	85	76	--
Liver: Hepatocellular Adenoma or Carcinoma[b]	3/14 (21)	4/34 (12)	1/32 (3)
P Values[c,d]	P = 0.046 (N)	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.549	0.146
Lower Limit		0.112	0.003
Upper Limit		3.399	1.668
Weeks to First Observed Tumor	85	76	102

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed Tolbutamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	0/12 (0)	1/30 (3)	0/18 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.023	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	83	--

[a] Treated groups received doses of 25,000 or 50,000 ppm in feed.

[b] Number of tumor-bearing animals/number of animals examined at site (percent).

[c] Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$, otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d] A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e] The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f] The 95% confidence interval of the relative risk between each treated group and the control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Tolbutamide in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma[b]	0/12 (0)	5/34 (15)	0/34 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.012		
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.487	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	79	--
Liver: Hepatocellular Adenoma[b]	0/12 (0)	2/34 (6)	1/34 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.112	0.020
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	103	103

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Tolbutamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	1/12 (8)	0/34 (0)	0/34 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		6.492	6.492
Weeks to First Observed Tumor	103	--	--
Liver: Hepatocellular Adenoma or Carcinoma[b]	1/12 (8)	2/34 (6)	1/34 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.706	0.353
Lower Limit		0.042	0.005
Upper Limit		40.714	26.851
Weeks to First Observed Tumor	103	103	103

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Tolbutamide in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Carcinoma[b]	0/11 (0)	2/26 (8)	0/26 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.138	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	83	--
Pituitary: Chromophobe Adenoma or Carcinoma[b]	1/11 (9)	2/26 (8)	0/26 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.846	0.000
Lower Limit		0.052	0.000
Upper Limit		47.845	7.805
Weeks to First Observed Tumor	103	83	--

[a] Treated groups received doses of 25,000 or 50,000 ppm in feed.

[b] Number of tumor-bearing animals/number of animals examined at site (percent).

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Tolbutamide in the Diet[a]

(continued)

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[c] Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$, otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d] A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e] The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f] The 95% confidence interval of the relative risk between each treated group and the control group.

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